CHANGES IN THE K AND Na CONTENT OF SCENEDESMUS OBTUSIUSCULUS

by

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Summary

We examined the K and Na content of *Scenedesmus obtusiusculus* at different external Na concentrations. The alga accumulates these ions, and the ratio of K/Na is 2 : 1, irrespective of the ion concentration of the external solution. However, if the K concentration of the medium is low (10^{-4} M), the cell is able to substitute Na for K to a certain extent; the K/Na ratio changes to 1 : 3.

A major part of the Na content of the cells can be removed by a Ca-Mg solution. Even after denaturation and washing with distilled water, some bound K and Na ions remain.

The cells containing a large amount of Na show a rapid net K accumulation, simultaneously with a Na efflux. As the flux rates show, however, the Na lost by the cells takes part in the K influx only to a limited extent, in greater part it is found in the easily replaceable Donnan places and in the double electrical layer.

At 0 C° no net K accumulation can be observed while the Na efflux decreases continuously.

Introduction

Analyzing the assimilation of unicellular freshwater algae, many authors think that the cells preserve a low inner Na concentration with the help of the Na extrusion mechanism (Barber 1968a, Kylin 1966). However, studies on the enzyme-system (transport ATPase) responsible for the K-Na substitution did not reveal any clear-cut connection between the enzyme and the transport of monovalent cations (Bonting–Caravaggio 1966, Erdei – Meszes 1970, Ishida – Mizushima 1969, Sexton – Sutcliffe 1969).

Unicellular and higher plants, however, often accumulate Na as well, and in these species it does not make any sense to speak about a Na

extrusion mechanism. The very heterogeneous data referring to the transport ATPases of plants are most probably based on such physiological difference.

In order to establish the inner K and Na concentration of the experimental objects, the mobility of the ion-reserves, and the rates of the net ion-transport compared with the fluxes by labelled ions in the steady-state, the present paper reports on experiments which were carried out with cells growing in nutrient solutions with high K and low Na, or high Na and low K concentrations.

Materials and Methods

The experiments were carried out with *Scenedesmus obtusiusculus* C H O D, a unicellular green algae, which was grown in a non-synchronous culture medium as described earlier (M e s z e s et al. 1967).

The K concentration of the modified K n o p – P r i n g s h e i m nutrient solution was 6,09 mM. There was no Na in the medium. The concentration of K and Na ions was varied according to Table I. In the other components of the nutrient there was no change.

Table I.

Nutrient solutions with different K and Na concentrations

Nutrient solution	Ion concentration, mM		Characteristics of the nutrient solution
	K	Na	
I.	6,09	–	Normal, without Na, (modified Knop-Pringsheim)
II.	6,09	6,09	Rich in K – Na
III.	–	6,09	Without K
IV.	6,09	0,1	Poor in Na
V.	0,1	6,09	Poor in K

The cells were obtained by centrifugation, and they were washed twice with distilled water before use. The experiments were done as follows:

1. The K-Na content the alga washed with distilled water was used as control.
2. The concentration of the Ca and Mg in solution was 0,3, and 0,2 mM, respectively, the same values as the concentrations of these ions in the nutrient solution. The exposure time was 2 hours in the Ca-Mg solution. Then the cells were washed five times.
3. The denaturation of the cells was done by boiling in distilled water for 5 minutes. The cells were then washed five times with distilled water.
4. The uptake and the release of K-Na took place in a 5 mM KNO_3 or 5 mM $NaNO_3$ solution for 15, 30 and 120 minutes.

5. The experiments were carried out at room temperature except where otherwise stated (0° C).
6. The concentration of the applied ouabain was 1 mM.
7. The experiments were made in diffuse light of low intensity.

After treatment aliquots of the material were dried at 80° C and then reduced to ashes at 450° C for 45 minutes. After that the samples were dissolved in 3N HCl, and their K and Na content determined by flame photometry.

The K and Na content of the cells is expressed at μ-equiv/ml cell volume. The volume of the cells may determined by hematocryt-tubes supplemented by simultaneous cell counting. The extracellular space of the cenrifuged cells was not measured, therefore this factor was not taken in consideration. The wet and dry weights per unit volume of the alga cells was also determined.

Results

The internal ion-content of the algae cells

The cells were grown in nutrient solutions of various K and Na concentrations as mentioned above. As shown in Fig.1, the K/Na ratio of the cells growing in nutrient solutions I, II, and IV is 1 : 2 (column Ia, IIa and IV a). If the K and Na concentration of the medium is equal, (IIa.) the internal ratio is unchanged, but the ion content increases slightly.

After some cell divisions in solution III – which contains no K – the algae do not multiply, therefore there are no data available. (When the high exogen Na concentration is supplemented by a minimum quantity

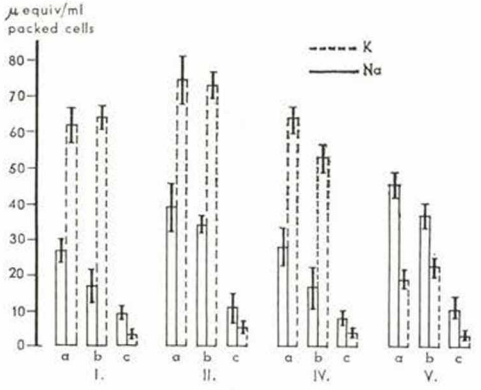

Fig. 1. The internal K (– – –) and Na (———) content of cells grown in different nutrient solutions (I – V.) Control (a), washed with Ca-Mg solution (b), and washed with distilled water after denaturalization (c). Average of 6 experiments.

of K (10^{-4} M, nutrient solution V), the culture grows at normal speed, but the original K : Na = 2 : 1 ratio is reversed, and the K : Na ratio of the starved cells becomes 1 :3 (Va).

The K content of the cells is but slightly influenced by divalent cations (0,3 mM Ca + 0,2 mM Mg.) Upon treatment with a Ca-Mg solution for 2 hours there is no, or only a slight change in the K content, (Fig. 1. Ib, IIb, IVb, Vb) while the internal Na concentration of the algae grown in a Na deficient nutrient solution decreases by 40% ,and that of the algae grown in a Na rich nutrient solution decreased by about 15 – 20% (IIb, IVb).

After denaturation and washing with distilled water the ion-content of the cells grown in any of the nutrient solutions tested decreases to a low level. There remains generally 8 – 10 mM sodium and 3 – 4 mM potassium in the cells (Fig. 1. Ic, IIc, IVc, Vc).

The results on the Na content of cells grown under different conditions are shown in Table II.

Table II.

The distribution of the Na content in cells grown in nutrient solutions of different Na concentrations

Nutrient solution	Released upon washing with Ca – Mg		Released upon Denaturation		Stable	
	μeq/ml	%	μeq/ml	%	μeq/ml	%
Deficient in Na						
I.	10,0	37,7	8,1	30,5	8,4	31,6
IV.	11,4	41,4	8,6	31,2	7,5	27,2
Rich in Na						
II.	5,6	14,3	23,4	59,8	10,1	25,8
V.	8,5	18,8	26,7	59,2	9,9	21,9

The results in Table II suggest that the Na content of the cells can be devided into three, well defined parts:

Na ions released upon washing with divalent cations
Na ions released after denaturation
Na ion bound in a stable form

The Na content that can be released by washing with divalent cations is higher in the cells grown in a nutrient solution deficient in Na than in cells grown in the presence of higher Na concentrations. The higher Na content of the cells grown in nutrient solutions rich in Na is released after denaturation and becomes localized in the fraction which can not be removed by washing with Ca-Mg. Here the Na concentration is three times higher than in a similar fraction of the cells grown in Na-deficient nutrient solutions.

Changes in the ion-concentrations of cells with a normal high K and low Na content

As shown above, if the K concentration of the external medium is high enough, the K content of the cells will be several times higher than their Na content irrespective of the Na concentration. If cells with such

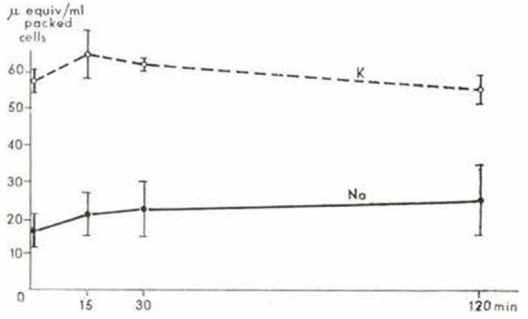

Fig. 2. Changes in the K(– – –) and Na (———) content of normal cells with a high K and a low Na content. The cells were in a 5 mM KNO_3 solution. Average of 4 experiments.

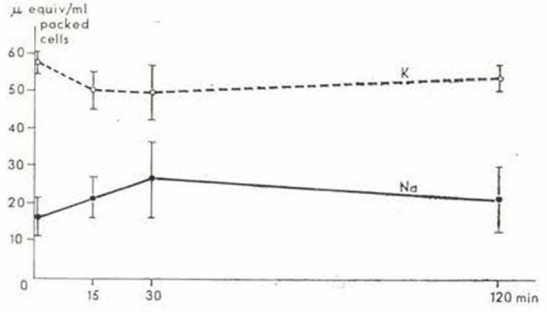

Fig. 3. Changes in the K(– – –) and Na(———) content of normal cells with a high K and a low Na content. The cells were in a 5 mM $NaNO_3$ solution. Average of 4 experiments.

a high K concentration are placed in 5 mM KNO_3 solution for periods of various lengths, there is practically neither net accumulation, (Fig. 2) nor induction of Na efflux. In 5 mM $NaNO_3$ a K-Na exchange occurs in the first 30 minutes, but after 120 minutes the original state is restored (Fig.3).

Changes in the ion-concentration of cells with a low K and a high Na content

In further experiments K-starved cells (Nutrient solution V) were used.

The K deficient cells show a rapid net accumulation from a 5 mM KNO_3 solution. The process of accumulation will stop in 30 minutes

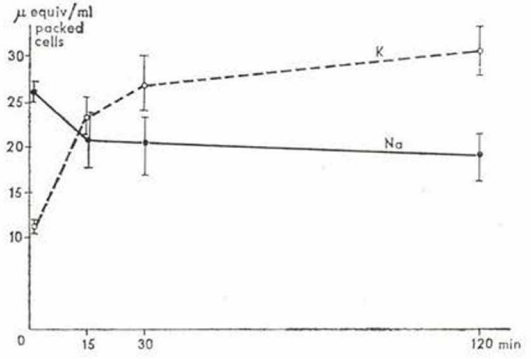

Fig. 4. K accumulation (— — —) and Na (———) relase of K-deficient cells in a 5 mM KNO_3 solution. Average of 8 experiments.

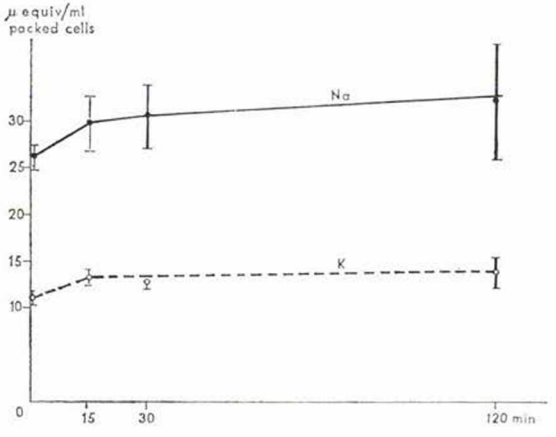

Fig. 5. Changes in the K (— — —) and Na (———) content of K-deficient cells in 5 mM $NaNO_3$ solution. Average of 4 experiments.

(Fig. 4). In its linear phase the rate of flux is associated with an uptake of 1 pmole K/cm² sec, and release of 1,4 pmole Na/cm² sec.

In a 5 mM $NaNO_3$ solution, the Na content of the cells inreases sligtly as function of time, the K concentration remains at the same level (Fig. 5).

The K uptake by the K deficient cells is inhibited at O° C and a gradual loss of Na takes place (Fig. 6).

The presence of 1 mM ouabain has no influence on either process, a few per cent inhibition may occur (Fig. 7). The data of the two latter figures regarded as preliminary results, because of the limited number of experiments.

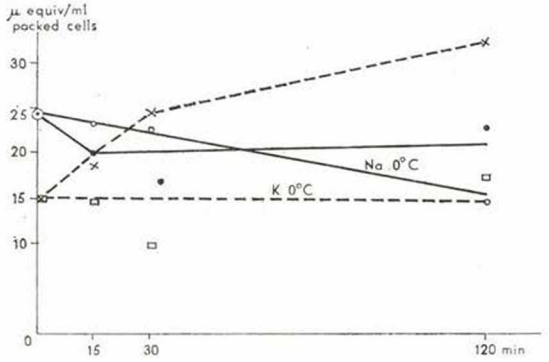

Fig. 6. Lack of K uptake of K deficient cells at 0 °C (□ – – – □). Changes in Na content are marked by (o——o). Changes in K (x– – –x) and Na (.——.) are shown at 21 °C (control).

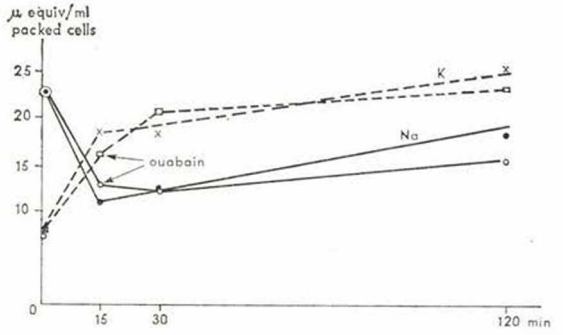

Fig. 7. Influence of 1 mM ouabain on K uptake (□ – – – □) and Na release (o——o) of K-deficient cells. Control of K intake (x– – –x) and Na loss (·– – –·). 1 experiment.

Discussion

The ion-content of the cells, and their exchange by divalent ions

According to the results obtained in the first part of the experiments, there is no or only a slight influence of the Na concentration of the nutrient solution on the Na content of the cell. The production of deficient

cells was not successful, the endogenous Na concentration did not get below 10 μequiv Na/ml alga-cell.

We succeeded in producing K deficient cells, and in agreement with the results of Barber (1968a), the Na content of such cells increased and the K/Na ratio was reversed.

The internal ion-concentration of the cells was 10 times higher than the ion-concentration in the external medium. The K-starved cells accumulated their 10^{-2} M internal K concentration from a medium containing 10^{-4} M K. This is even more expressed in the case of Na ions, because Na ions can be accumulated to an internal concentration of 10 to 20 mM from the Na impurities of the external medium. In the presence of such a low external K concentration, the cells did not grow (e.g. nutrient solution III).

According to Schaedle and Jacobson (1965, 1966) K taken up during a short time can completely be replaced by divalent ions, but a certain percentage of the accumulated K, about 16 – 24 μequiv/100 g dry weight can no more be washed out, if uptake goes on for a long time. In their opinion the exchangeable ions are most probably found on the surface of the cells.

As our own experiments indicate, in a Ca – Mg solution of low concentration, 21 – 41% of the Na content of the cells can be exchanged. The time dependence of this process was not been studied in detail. According to literary data, however, the labile Na fraction is washed out quickly, generally within 1 minute (Schaedle – Jacobson 1965), perhaps in 10 – 15 minutes (Collins – House 1969). Though in our experiments labile Na was released as a result of a 120 – 150 minute washing with Ca – Mg solution, our experimental results concerning K uptake show that the release of Na takes place in 15 minutes, and after a longer exposure time the Na content of the cells changes very little if at all. Therefore we can suppose that the loss of Na caused by divalent ions takes place in a similarly short time.

After the denaturation of the cells 30 – 60% of the Na content could be washed out with distilled water, and there remained about 10 μequiv Na/ml alga cells firmly bound in the cells, i.e. about 20 – 30% of the total Na content. It is quite possible that the Na and K that remained bound after denaturation do not take part in the formation of the free ion-concentration and consequently in that of the membrane potencial. According to the sorption-theory this quantity is bound (Spanswick 1968).

The results of our experiments do not explain why, as shown in Table II, the amount of labile Na ions is larger in cells grown in Na-deficient media than in those grown in media rich in Na ions. The data of Table II show that the higher Na content of the cells grown in the presence of high Na concentration is due to a several fould increase in the plasmatic Na fraction which is released after denaturation.

Net K accumulation and concomitant phenomena

In the uptake of K several parallel mechanisms are involved. In algae and higher plants K influx was found to be influenced, or be the result of several other phenomena.

a) anion influx: Cl^- (Barber 1968c, Jeschke 1970, Nobel 1969) and HCO_3^- (Findlay et al. 1969);
b) hydrogen efflux (Nobel 1969);
c) depolarization or hyperpolarization of the membrane (Jeschke 1970);
d) changes in the K permeability of the membrane (Jeschke 1970);
e) Na efflux (Barber 1968c, Kylin 1966, Nobel 1969);
f) changes in the stoichiometry of ATP—K influx (Jeschke 1970);

Without a simultaneous measurement of the conditions of the anion transport the estimation of the nature of net K accumulation, as shown in Fig. 4, is difficult. Simultaneously with the net accumulation of K at a rate of 1 pmole/cm² sec there is a release of Na at a rate of 0.4 pmole/cm² sec. In accordance with Barber's results (1968a), the Na efflux in *Chlorella* cells, containing a considerable amount of Na, could be stimulated by the administration of 1 mM external K, a combination, at least in part, of the fluxes of these two ions, seems therefore possible. Our results apparently also indicate that 40—50 per cent of the K influx connected with Na efflux.

A comparison of the speed of net ion flux measured by conventional chemical methods with the speed of isotope exchange measured in the steady-state points to a connection between the real ion flux and the permeability of the membrane. Relying upon these considerations Schaedle—Jacobson (1966) concluded that the K accumulation in Chlorella is limited by the permeability of the membrane, since both the steady-state change measured by using K^{42} and the net accumulation amounted to 13 μequiv/100 g dry weight. At the same time in the case of Na permeability was not the limiting factor because while the steady-state Na diffusion was 90 μequiv/100 g dry weight, net accumulation amounted only to 18—20 μequiv/100 g dry weight.

Similar results were obtained with K. The speed of K flux measured in the steady-state was 1 pmole/cm² sec in light and 0,5—0,7 pmole/cm² sec in darkness. Accordingly the speed of net accumulation was 1 pmole/cm² sec.

The 0,4 pmole/cm² sec speed of the Na efflux accompanying K accumulation can no more be parallelled with the steady-state change, the speed of which was ten times lower, 0,04 pmole/cm² sec (Meszes at al. 1967) as calculated from Fig. 6. The speed of Na flux is thus limited by the permeability of the membrane and so only about 1/10 of the efflux can permeate the membrane. The remaining major part probably belongs to the fraction of labile Na. This conclusion is supported by the fact

that the amount of Na released in 120 minutes in the presence of K accumulation (5—8 μequiv/ml alga-cells is equal to that amount which can be washed out in 120 minutes by Ca—Mg solution (7 μequiv/ml alga-cells). Thus it seems probable that K influx is connected with Na efflux only to a small extent and the Na released derives from the Donnan free space and from the double electrical layer where it can be easily substituted by divalent or other monovalent cations. Since the cells had previously been washed out with distilled water the free space of water can not be taken into consideration.

In contrast to the inhibition of K efflux at 0 °C, Na efflux decreases only gradually and none of the processes is inhibited by ouabain to a considerable extent. Some inhibition of a few per cent may, of course, be at work. Our results concerning the above finding should be regarded as preliminary, because of the limited number of experiments. The lack of any considerable inhibition by ouabain also indicates the lack or very scarce participation of the combined K—Na transport system.

Speeds of K and Na fluxes

It is remarkable that the speed of K flux is nearly identical in various living organisms. The speed of K influx in Chara corallina is 1 pmole/cm² sec (Findlay et al. 1969) in Nitella 1—1,5 pmole/cm² sec (Walker — Hope 1969), in Chlorella 1 pmole/cm² sec in light and 0,18 pmole/cm² sec in darkness (Barber 1968b), in Scenedesmus 1 pmole/cm² sec according to our own measurements.

On the basis of the concentration dependence of ouabain inhibition Solomon and Glynn (Solomon et al. 1956, Glynn 1957) estimated the number of active places responsible for the K—Na transport in erythrocytes as being 10^3—10^4 such places on the surface of the whole membrane.

Calculating with a turnover rate of 100 (Stein 1968) on the basis of a flux speed of 1 pmole/cm² sec, the number of the K transport places for a cell 5 μ wide, 10 μ long and with a surface of 170 μ^2 is:

$$A = \frac{N_A \varnothing a}{10^8 n} = \frac{6 \cdot 10^{23} \cdot 1 \cdot 10^{-12} \cdot 170}{10^8 \cdot 100} = 10^4$$

where: N_A = Avogadro-number: $\varnothing$ = the speed of the flux (M/cm² sec)
a = cell-surface in μ^2 n = turnover number 10^8 = key-number (1 cm² = 10^8 μ^2).

The speed of Na influx and efflux is generally lower, in Chara 0,2—0,5 pmole/cm² sec (Findlay et al. 1969), in *Nitella* 0,1 (McRobbie — Enid 1962), or 0,04—0,06 pmole/cm² sec according to other sources (Walker — Hope 1969), 0,18 pmole/cm² sec in *Chlorella* according to Barber (1968a). With *Scenedesmus* our own

measurements gave 0,4 pmole/cm² sec as the speed of net Na efflux, but on the basis of isotope exchange, with no net ion uptake the result was only 0,04 pmole/cm² sec.

Na flux speeds are generally 5—10 times lower, than K flux speeds and they are much more variable. This phenomenon also indicates that a part of the Na is highly labile in the metabolism and plays a different role than K, but can replace it at readily accessible sites.

Possible mistakes affecting the experimental results

The relatively great standard deviation of the results is caused by the qualitatively identical, but quantitatively different values obtained in the experiments carried out at different times. This often happens if the experiments are carried out with biological objects. Under appearently identical conditions deviations as high as 50 to 100 per cent may occur.

While comparing the results of different authors, the main difficulty consisted in finding a common basis for the data expressed in different dimensions by various authors. In our own experiments ion content was expressed as concentration (mM = μequiv/ml alga cell), flux speeds as pmole ion/cm² sec.

In the calculation of the concentrations the extracellular water-space of the centrifuged alga cells was not taken into consideration for want of data concerning our own object. On the basis of the dilution of labelled mannitol this value was 33% in the case of 1 ml *Chlorella*. Therefore our experimental results are lower than the real values.

REFERENCES

Barber, J. 1968. Sodium efflux from Chlorella pyrenoidosa. Biochim. Biophys. Acta **150:** 730—733.

Barber, J. 1968b. The influx of potassium into Chlorella pyrenoidosa. Biochim. Biophys. Acta **163:** 141—149.

Barber, J. 1968c. Light-induced uptake of potassium and chloride by Chlorella pyrenoidosa. Nature **217:** 877—878.

Bonting, S. L. — Caravaggio, L. L. 1966. Studies on Na-K-activated adenosine triphosphatase. XVI. Its absence from the cation transport system of Ulva lactuca. Biochim. Biophys. Acta. **112:** 519—523.

Collins, J. C. — House, C. R. 1969. The exchange of sodium ions in the root of Zea mays. J. Exp. Bot. **20:** 497—506.

Erdei, L. — Meszes, G. 1970. ATPáz aktivitás a Scenedesmus sp. zöld algában. XI. Biokémiai Vándorgyűlés előadásai **III:** 86. Sopron.

Findlay, G. P. — Hope, A. B. — Pitman, M. G. — Smith, F. A. — Walker, N. A. 1969. Ionic fluxes in cells of Chara coralina. Biochim. Biophys. Acta **183:** 565—576.

Glynn, I. M. 1957. The action of cardiac glycosides on sodium and potassium movements in human red cells. J. Physiol. **136:** 148—173.

Ishida, M. — Mizushima, S. 1969. Membrane ATPase of Bacillus megatherium. I. Properties of membrane-ATPase and its solubilized form. J. Biochem. **66:** 33—43.

Jeschke, W. D. 1970. Der Influx von Kaliumionen bei Blättern von Elodea densa, Abhängigkeit vom Licht, von der Kaliumkonzentration und von der Temperatur. Planta **91:** 111 – 128.

Kylin, A. 1966. Uptake and loss of Na, Rb, and Cs in relation to an active mechanism for extrusion of Na in Scenedesmus. Plant Physiol. **41:** 579 – 584.

MacRobbie, E. A. – Enid, A. C. 1962. Ionic relations of Nitella translucens. J. Gen. Physiol., **45:** 861 – 878.

Meszes, G. – Kralovanszky, J. – Cseh, E. – Böszörményi, Z. 1967. Ion and amino absorption by unicellular algae. Acta Biochim. Biophys. Acad. Sci. Hung. **2:** 239 – 251.

Nobel, P. S. 1969. Light dependent potassium uptake by Pisum sativum leaf fragments. Plant and Cell Phys. **10:** 597 – 605.

Schaedle, M. – Jacobson, L. 1965. Ion absorption and retention by a Chlorella pyrenoidosa. Plant Phys. **40:** 214 – 220.

Schaedle, M. – Jacobson, L. 1966. Ion absorption and retention by Chlorella pyrenoidosa. II. Permeability of the cell to sodium and rubidium. Plant Phys. **41:** 248 – 254.

Sexton, R. – Sutcliffe, J. F. 1969. Some observations on the characteristics and distribution of AtPases in young roots of Pisum sativum cv. Alaska. Annals of Botany, **33:** 683 – 694.

Solomon A. K. – Gill, T. J. – Gold, G. L. 1956. The kinetics of cardiac glycosida inhibition of potassium transport in human erythrocites. J. Gen. Physiol. **40:** 327 – 350.

Spanswick, R. M. 1968. Measurements of potassium ion activity in the cytoplasm of the Characeae as a test of the sorption theory. Nature, **218:** 357.

Stein W. D. 1968. Turnover numbers of membrane carriers and the action of the polypeptide antibiotics. Nature **218:** 570 – 571.

Walker, N. A. – Hope, A. B. 1969. Membrane fluxes and electric conductance in Characean cells. Austr. J. Biol. Sci. **22:** 1179 – 1195.